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RELATION BETWEEN ABERRANT α-CATENIN EXPRESSION AND LOSS OF E-CADHERIN FUNCTION IN PROSTATE CANCER

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It is now well documented that E-cadherin expression correlates inversely with tumor grade in various carcinomas *including prostate cancer.* We also demonstrated a statistically significant correlation between decreased E-cadherin expression and progression-free period in early stage patients treated by radical prostatectomy and decreased survival in patients with advanced stage disease. We now study the relationship between E cadherin and α-catenin expression, because in prostate cancer cell lines, mutational inactivation of the α-catenin gene can be the cause of the impaired E-cadherin function. Twenty patients treated by radical prostatectomy and 32 advanced stage patients were evaluated immunohistochemically for E-cadherin and α-catenin expression. The results were related to tumor grade and disease progression. Four patients in the radical prostatectomy group had aberrant E-cadherin and α-catenin expression and showed disease progression. The other 16 patients were free of progression and had normal E-cadherin and α-catenin expression. In the advanced stage group, 4 of 13 patients with normal E-cadherin staining showed aberrant α-catenin expression and 2 patients (50%) progressed, compared with only 22% progression in patients with both normal E-cadherin and α-catenin expression. The other 19 patients with aberrant E-cadherin and α-catenin staining had the poorest prognosis. *Our results suggest that loss of α-catenin expression could be* one of the mechanisms responsible for the loss of E-cadherin–mediated cell-cell adhesion in human prostate cancer and might in some cases provide prognostic information. *Int. J. Cancer* 74:374–377, 1997.
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Prostate cancer is well known as a disease with an unpredictable biological potential. Not every patient with prostate cancer will progress (Adolfsson *et al.,* 1990; Johansson *et al.,* 1989); on the other hand, metastases apparently still develop in a considerable group of patients who are treated radically for their localized disease (Bostwick *et al.,* 1993). Tumor grade and clinical stage have been so far used as prognostic factors, although the pathologic stage appears to have the best prognostic value (George, 1988; Grayhack and Assimos, 1983; Morton *et al.,* 1991). Therefore, many studies have been performed to define new prognostic markers, and one of these is E-cadherin, an epithelial cell adhesion molecule (Takeichi, 1988).

Loss of E-cadherin expression has been shown to correlate with an *in vitro* invasive phenotype of cancer cell lines (Bussemakers *et al.,* 1992; Frixen *et al.,* 1991). Furthermore, in human cancers (including prostate cancer), aberrant E-cadherin expression correlates significantly with increasing tumor grade (Umbas *et al.,* 1992). We have found significant correlations between aberrant E-cadherin expression and survival in patients with prostate cancer (Umbas *et al.,* 1994). The prognostic value appeared to be independent of tumor stage, because E-cadherin immunohistochemistry kept its prognostic value when we stratified the results according to clinical stage, *i.e.,* patients with localized disease and those with tumors that extended beyond the prostatic capsule (Umbas *et al.,* 1994). However, not every patient with normal E-cadherin expression had a better survival. This suggests that there might be mechanisms in the cancer cells that suppress E-cadherin function even in the presence of the protein as demonstrated immunohistochemically (Oka *et al.,* 1992; Shimoyama and Hirohashi, 1991). Morton *et al.* (1993) have shown that loss of normal E-cadherin function in prostate cancer cell lines can occur through mutational inactivation of the α-catenin gene. Kadowaki *et al.* (1994) have shown that α-catenin immunohistochemistry has additional prognostic value for esophageal carcinoma. These findings prompted us to investigate the relationship between E-cadherin and α-catenin expression in prostate cancer patients.

MATERIAL AND METHODS

Patients

Twenty early stage prostate cancer patients and 32 advanced stage patients were included in our study. Treatment options were radical prostatectomy for early stage and hormonal treatment for advanced disease. Follow-up was done by measuring the prostate-specific antigen level, and bone scan was performed to detect metastatic lesions.

Surgical specimens

All tissues were obtained at the time of surgery, either radical prostatectomy or transurethral resection, and were snap frozen. Four- to 6-μm serial sections from the frozen tissues were cut on a cryostat, air dried, and stored at −20°C until use. One section from each patient was stained with hematoxylin and eosin to determine the hispathologic grading using the Gleason score system (Gleason, 1977).

Antibodies

Uvomorulin (L-CAM) monoclonal antibody (MAb) against E-cadherin (Eurodiagnostica, Apeldoorn, The Netherlands) and α-18, a rat MAb against α-catenin (Shimoyama *et al.,* 1992), a kind gift from Dr. S. Hirohashi (Tokyo, Japan), were used in this study.

Immunohistochemistry

Immunohistochemistry was performed using an indirect method as described previously (Umbas *et al.,* 1992), except that we used biotinylated (Amersham, Aylesbury, UK) anti-mouse Ig (E-cadherin) and anti-rat Ig (α-catenin) staining as secondary antibody and incubated with avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector, Burlingame, CA) before incubation with diaminobenzidine 0.6 mg/ml in 0.65% imidazol/phosphate-buffered saline.

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E-cadherin and α-catenin staining patterns were scored as described previously (Schipper *et al.*, 1991; Umbas *et al.*, 1992). Uniformly positive staining was regarded as normal, whereas heterogeneous, uniformly negative and cytoplasmic staining were scored as aberrant.

RESULTS

As earlier reported, there was a significant correlation between E-cadherin expression and tumor grade (Umbas *et al.*, 1992). In clinical follow-up, we found that E-cadherin staining was significantly correlated with tumor stage and survival in the advanced stage patients and with progression-free interval in patients with low stage disease treated by radical prostatectomy (Umbas *et al.*, 1994). In this study, we found a significant correlation between α-catenin and E-cadherin expression (Table I; $\chi^2 = 38.2$, $p < 0.001$). Nevertheless, 3 staining combinations could be de-

TABLE I – CORRELATION BETWEEN E-CADHERIN AND α-CATENIN EXPRESSION[1]

	α-Catenin	
	Normal	Aberrant
E-cadherin		
Normal	25	4
Aberrant	0	23

[1] $\chi^2 = 38.2$, $p < 0.001$.

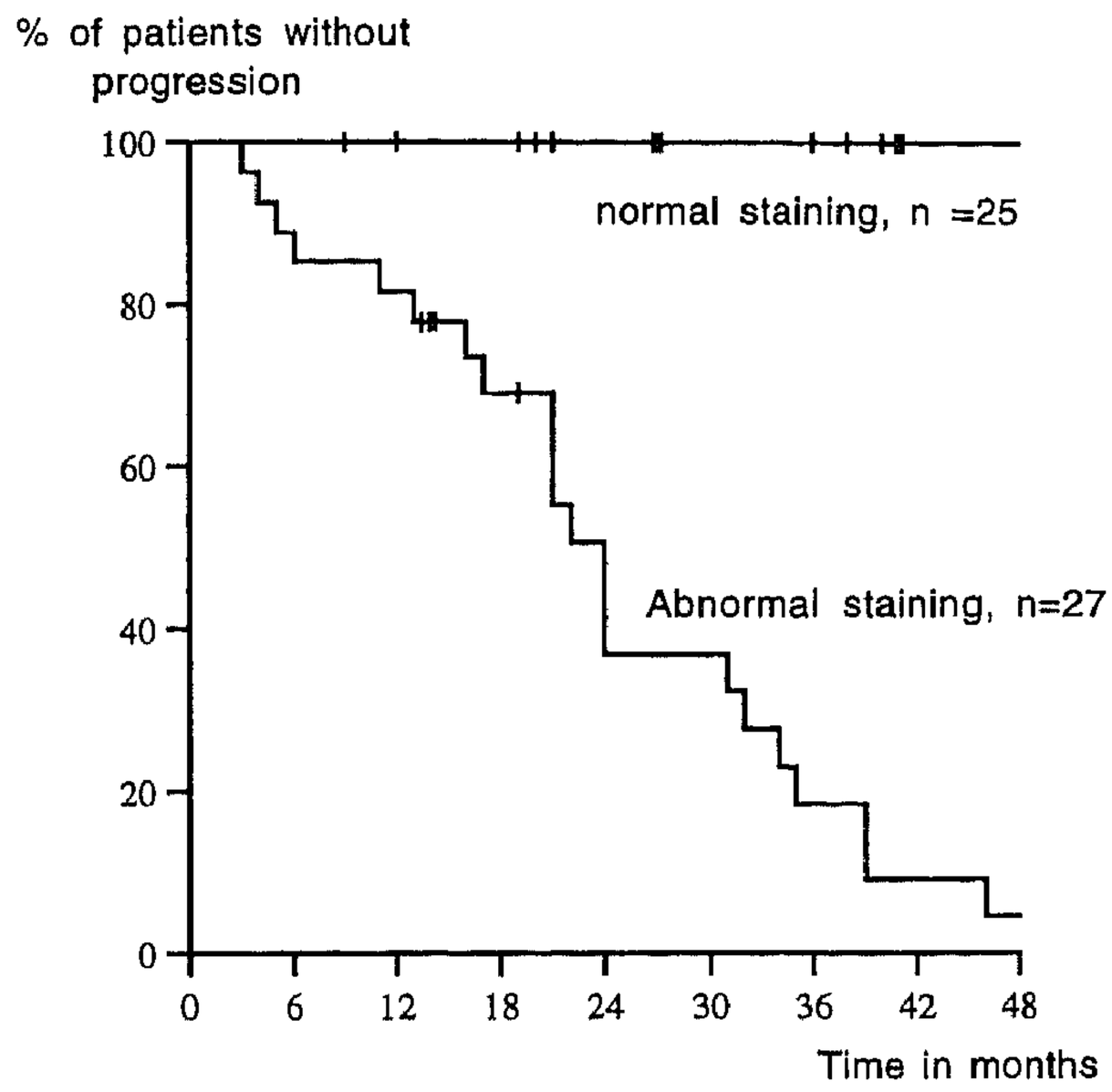

FIGURE 2 – Kaplan–Meier 4-year survival curves according to α-catenin expression ($\chi^2 = 48.8$ by log rank test, $p < 0.001$).

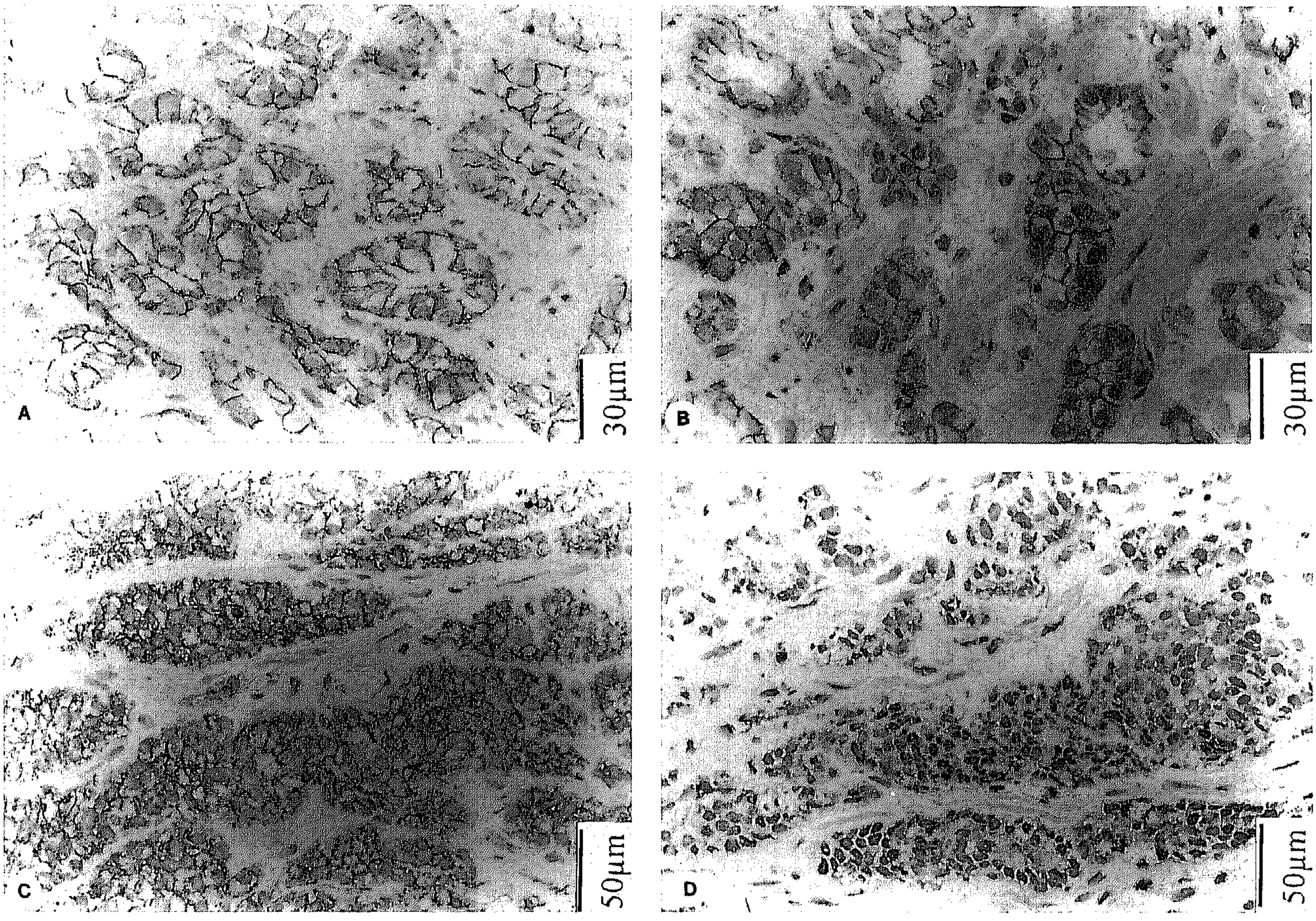

FIGURE 1 – Immunohistochemical analyses of 2 representative prostate cancer specimens with anti–E-cadherin *(A, C)* and anti-α-catenin (α 18; *B,D*). Note the concordance in staining in A and B, whereas α-catenin is heterogeneous (D) despite normal E-cadherin immunoreactivity *(C)* in the other sample. Scale bar: 10 μm.

TABLE II – CORRELATION BETWEEN E-CADHERIN AND α-CATENIN IN ADVANCED CASES (n = 32)

	Patient outcome	
	Progression (−)	Progression (+)
Normal E-cadherin and α-catenin	7 (78%)	2 (22%)
Normal E-cadherin and aberrant α-catenin	2 (50%)	2 (50%)
Aberrant E-cadherin and α-catenin	3 (16%)	16 (84%)

fined: first, normal expression of both E-cadherin and α-catenin (Fig. 1A,B); second, normal E-cadherin expression and aberrant α-catenin (Fig. 1C,D); and third, both aberrant expression of E-cadherin and α-catenin (data not shown). Taken together, patients who showed normal immunoreactivity for both of these molecules had significantly higher 4-year survival rates than patients having aberrant α-catenin expression even if the E-cadherin expression was normal (Fig. 2; $\chi^2 = 48.8$ by log rank test, $p < 0.001$).

In the radically treated group, 4 patients showed aberrant E-cadherin and α-catenin expression; all these patients progressed within 8 to 37 months. The other 16 patients of this group showed normal expression of both E-cadherin and α-catenin, and no progression could be detected during a mean follow-up of 40 months (range: 20–65 months).

Thirteen patients with advanced disease had normal E-cadherin expression. Nine of these patients also had normal α-catenin expression, and 2 (22%) died of disease; 2 of 4 patients (50%) in this group who had aberrant α-catenin expression died of prostate cancer (Table II). Of the other 19 patients who had both aberrant expression of E-cadherin and α-catenin, only 3 (16%) were alive without progression, and the other 16 (84%) either showed progression (1 patient) or died of disease.

DISCUSSION

The use of E-cadherin immunohistochemistry has gained considerable attention for the diagnosis and prognosis of carcinomas (Birchmeier and Behrens, 1994). We have reported that decreased expression of this epithelial cell-cell adhesion molecule is indicative of a poor clinical course of the disease (Umbas *et al.*, 1994). In an attempt to establish the mechanisms associated with impaired cadherin function, Morton *et al.* (1993) found that α-catenin, a protein essential for linkage to the cytoskeleton, was absent due to homozygous deletion of the gene. Evidence that this could have implications for diagnosis was first reported by Kadowaki *et al.* (1994), *i.e.*, a group of tumors with a normal E-cadherin pattern showed an absence of α-catenin. In our study, we also found tumors homogeneously positive for E-cadherin, despite the absence of immunoreactivity against α-catenin, albeit this cohort was much smaller than that reported for esophageal carcinomas (Kadowaki *et al.*, 1994). In the cohort of patients who were studied, only 4 of 39 tumors that appeared to be normal for E-cadherin showed aberrant expression of α-catenin. Interestingly, 2 of these had poor prognosis, *i.e.*, clinical progression after radical prostatectomy. Importantly, all of the cases showing abnormal E-cadherin patterns were also scored likewise for α-catenin. Although the numbers are small, this suggests that α-catenin immunohistochemistry might have additional prognostic value and may even replace E-cadherin in this respect. However, due to the experimental uncertainties of immunohistochemistry analyses, the combined use of both antibodies appears to be recommended. We thus conclude that in human prostate cancer, α-catenin deficiency may indeed be related to defective cadherin function and associated poor prognosis. However, the high concordance between α-catenin and E-cadherin immunohistochemistry suggests that this is only the case in a minority of cases. The mechanisms underlying this lack of α-catenin staining despite normal E-cadherin expression have not been resolved. The loss of α-catenin can be causally related to impaired cadherin function, *i.e.*, reintroducing neural α-catenin in the PC9 carcinoma cells lacking α-catenin, restores the calcium-dependent adhesiveness, and results in reversion of morphology (Hirano *et al.*, 1992). Hence, it is tempting to speculate that α-catenin can indeed function as an invasion suppressor gene in a subset of prostate cancer patients. Clearly, experimental evidence for this should be obtained by detailed molecular genetic analysis of the α-catenin gene in these cases. We have found that in the prostate, more than one cadherin subtype is responsible for cadherin-mediated interactions (data not shown). If we assume that the cadherin subtypes expressed in prostate cells all use α-catenin for anchorage to the cytoskeleton, this would mean that loss of α-catenin is indeed specific for loss of cadherin-mediated interactions. The low extent of diversity among α-catenins (only 2 are known, αE, and αN, 2 isoforms) further supports this hypothesis. Because our comparative immunohistochemical analysis of E-cadherin and α-catenin confronted with the clinical data also indicates that α-catenin is specifically associated with poor prognosis, we conclude that our method is preferable as a prognostic marker for studying prostate cancer.

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